CELL THERAPY FOR INTERVERTEBRAL DISC REPAIR: ADVANCING CELL THERAPY FROM BENCH TO CLINICS

L.M. Benneker[1,8], G. Andersson[2], J.C. Iatridis[3,8], D. Sakai[4,8], R. Härtl[5], K. Ito[6] and S. Grad[7,8,*]

[1]Department of Orthopaedic Surgery, University of Bern, Bern, Switzerland
[2]Department of Orthopaedic Surgery, Rush University Medical Center, Chicago, IL, USA
[3]Department of Orthopaedics, Mount Sinai Medical Centre, New York, NY, USA
[4]Department of Orthopaedic Surgery, Tokai University School of Medicine, Isehara, Japan
[5]Department of Neurosurgery, Weill Cornell Medical College, New York, NY, USA
[6]Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands
[7]AO Research Institute Davos, Davos, Switzerland
[8]Collaborative Research Partner Annulus Fibrosus Repair Programme, AO Foundation, Davos, Switzerland

Abstract

Intervertebral disc (IVD) degeneration is a major cause of pain and disability; yet therapeutic options are limited and treatment often remains unsatisfactory. In recent years, research activities have intensified in tissue engineering and regenerative medicine, and pre-clinical studies have demonstrated encourageing results. Nonetheless, the translation of new biological therapies into clinical practice faces substantial barriers. During the symposium "Where Science meets Clinics", sponsored by the AO Foundation and held in Davos, Switzerland, from September 5-7, 2013, hurdles for translation were outlined, and ways to overcome them were discussed. With respect to cell therapy for IVD repair, it is obvious that regenerative treatment is indicated at early stages of disc degeneration, before structural changes have occurred. It is envisaged that in the near future, screening techniques and non-invasive imageing methods will be available to detect early degenerative changes. The promises of cell therapy include a sustained effect on matrix synthesis, inflammation control, and prevention of angio- and neuro-genesis. Discogenic pain, originating from "black discs" or annular injury, prevention of adjacent segment disease, and prevention of post-discectomy syndrome were identified as prospective indications for cell therapy. Before such therapy can safely and effectively be introduced into clinics, the identification of the patient population and proper standardisation of diagnostic parameters and outcome measurements are indispensable. Furthermore, open questions regarding the optimal cell type and delivery method need to be resolved in order to overcome the safety concerns implied with certain procedures. Finally, appropriate large animal models and well-designed clinical studies will be required, particularly addressing safety aspects.

Keywords: Regenerative medicine, clinical translation, intervertebral disc, cell therapy, barriers, patient selection, safety.

*Address for correspondence:
Sibylle Grad, PhD
AO Research Institute Davos
Clavadelerstrasse 8, 7270 Davos, Switzerland
Telephone Number: +41 81 414 24 80
FAX Number: +41 81 414 22 88
E-mail: sibylle.grad@aofoundation.org

Introduction

Intervertebral disc (IVD) degeneration is a major cause for neck and low back pain and as such a significant public health problem. According to the Global Burden of Disease Study, published in The Lancet in December 2012, low back pain is the leading cause of disability, with current estimates of 632 million people affected worldwide (Vos *et al*., 2012). In many cases neither conservative nor surgical treatment can provide satisfactory outcome for patients and clinicians. Hence there is a critical need for new therapies to attenuate disc degeneration and restore disc function. Research in the field of regenerative medicine and tissue engineering has intensified over the last decade, advocating attractive biomaterial, cellular and molecular solutions for IVD regeneration. Nonetheless, the translation of such new applications into clinical practice remains a major roadblock, and only few biological treatments have advanced to clinical studies. There are still serious hurdles to overcome before biological therapies for IVD repair can be introduced to the clinic. The aim of the symposium "Where Science meets Clinics", which was sponsored by the AO Foundation and held in Davos, Switzerland on September 5-7, 2013, was to address these barriers and to discuss strategies to overcome them. A diverse group of scientists, clinicians, health care industry and regulatory agency representatives was invited to present their views of current state of the art, major challenges and visions for advancement. This position paper focusses on the possibilities and difficulties of cell therapies for IVD regeneration. We first outline the present state of knowledge and then summarise the discussion among scientists and clinicians.

Cell Therapy in Intervertebral Disc Degeneration: Current State

In basic or pre-clinical research, one of three biological approaches is typically used to address the degenerative process: stimulating anabolic processes; modulating catabolic processes; and providing new cells. The latter approach appears very attractive, given that one important feature of IVD degeneration is a decrease in viable and functional cell numbers, and a substantial proportion of the existing cells are in a senescent state. IVD cells can broadly be separated in nucleus pulposus (NP) cells, which

are notochordal in the young and resemble chondrocytes in adults, and annulus fibrosus (AF) cells, which have fibroblast characteristics. The microenvironment, including mechanical and osmotic stresses and oxygen tension, has a significant effect on the cells, while IVD cells are generally characterised by low proliferative capacity.

Due to the limitations of differentiated IVD cells, different stem cell populations have been investigated for IVD regeneration. Evidence for the presence of endogenous stem/progenitor cells in the IVD has recently been accumulating; hence, mobilisation and activation of these cell pools appears an attractive strategy for enhancing self-repair (Sakai *et al*., 2012). To date, only *in vitro* data exist on the activation of endogenous stem/progenitor cells, and the amount of stem cells might be insufficient at the age when disc degeneration becomes a clinical problem. Mobilising stem cells from the surrounding tissues may be limited by the lack of blood supply. While results from organ culture studies have demonstrated the possibility of cell homing into degenerative discs (Illien-Junger *et al*., 2012), feasible applications of this promising finding in a clinical situation still need to be identified.

Alternatively, stem cells can be directly transplanted into the damaged disc. Numerous *in vivo* studies have been performed on the transplantation of mesenchymal stem cells (MSCs) in disc degeneration models. Bone marrow derived MSCs have been injected into rabbit (Sakai *et al*., 2003; Sakai *et al*., 2005; Sakai *et al*., 2006), rat (Crevensten *et al*., 2004), canine (Hiyama *et al*., 2008) and goat (Zhang *et al*., 2011) IVD, and largely demonstrated regenerative potential. Furthermore, xenogenic transplantation of human MSCs into porcine models (Henriksson *et al*., 2009) and injection of adipose tissue derived stem cells (Ganey *et al*., 2009) have also been reported. Promising outcomes have moreover been achieved with human disc cell or mesenchymal stem cell transplantations (Hohaus *et al*., 2008; Yoshikawa *et al*., 2010; Orozco *et al*., 2011) and with the delivery of cartilage cells (Acosta *et al*., 2011). Often the cells are delivered with a biomaterial carrier based on hydrogels such as hyaluronan or fibrin (Grad *et al*., 2010). Overall the results of these *in vivo* studies have demonstrated that MSCs are able to survive and proliferate after implantation into the disc and that they acquire phenotypic characteristics of IVD cells. In fact, co-culture of MSCs and IVD cells has widely been shown to induce an IVD-like phenotype in MSCs and stimulate new matrix production by the disc cells. Furthermore, there is substantial evidence that MSCs are able to suppress inflammatory reactions in the tissue. In spite of these promising results, there are serious concerns associated with MSC transplantation, including poor cell survival, cell leakage through injection site (Vadala *et al*., 2012), unintended differentiation towards osteogenesis, and the potential of tumourigenesis.

When considering cell therapy for IVD regeneration, its potential and limitations need to be identified. The underlying causes, such as the genetic predisposition, ageing, mechanics, smoking or obesity, cannot be addressed by cell therapy, and there are no *in vivo* data to suggest we can prevent disc degeneration in the long term. While much of the commonly observed disc degeneration may be a normal part of ageing, identification of pathological and painful conditions remains an active area of investigation, and it may be possible to slow or prevent such pathological disc degeneration. There are strong data suggesting that we can influence early degenerative changes. It is also evident that disc degeneration can lead to secondary degenerative spinal diseases, such as spinal canal stenosis, degenerative spondylolisthesis, facet joint osteoarthritis etc. It may be beneficial to apply cell therapy in patients with progressive disc degeneration before these diseases become advanced. The questions remain, at what stage of degeneration are we beyond biological repair and for how long can we halt disease progression?

To date only few clinical trials have been performed using cell therapy for IVD repair or regeneration. In the Euro Disc study, culture-expanded autologous disc cells were applied to patients with disc herniations. Two-year follow up data reported significant pain reduction, disc height preservation in the treated level, and maintenance of hydration in adjacent levels, which were evident only in the treatment group (Meisel *et al*., 2007). NuQu® is an innovative approach to disc repair that uses allogeneic juvenile (knee) chondrocytes with proven superior regenerative potential. The minimally invasive outpatient procedure targets disc-related pain and requires minimal patient rehabilitation. Studies in rat and porcine discs confirmed significantly superior magnetic resonance imageing (MRI) and histological outcomes in discs treated with the NuQu® system, in comparison to treatment with the fibrin carrier (Acosta *et al*., 2011). A phase I safety trial was subsequently performed with 15 patients with single level moderate lumbar disc degeneration (Coric *et al*., 2013). Continued improvement in pain scores as well as improved or unchanged MRI results could be observed from baseline to 6 and 12 months, warranting more extended human trials to assess efficacy. An on-going phase II clinical trial will assess the safety and effectiveness of NuQu® cartilage cell injection into the lumbar disc as compared to placebo in 44 subjects.

While animal and human data on the regenerative potential of injected chondrocytes or disc cells are promising, there are still open questions with respect to timing of treatment, the optimal cell source, cell pre-treatment, and cell carrier. According to current knowledge, the procedure appears safe; though long-term results are still unknown.

Cell Therapy for Disc Repair: Who is the Patient?

The most difficult question is to define the level of disc degeneration that is beyond biological repair and where an established surgical treatment is better indicated. Although it is obvious that regenerative treatment is best indicated at early stages of disc degeneration, prior to structural changes, these non-symptomatic discs usually are not seen by spine surgeons/therapists. Nevertheless, it is very likely that in the future, screening techniques focussing on genetic predisposition for early incidence of disc degeneration become available, and non-invasive imageing methods, such as quantitative MRI, are sensitive enough

to detect early degenerative changes. Several conditions were identified as candidates for cell therapy in disc repair.

Discogenic pain

Discogenic pain may originate from "black" discs or annular injured discs presenting HIZ (high intensity zones). In these cases, discogenic low back pain is believed to arise from acute tears or fissures of the AF and from focal defects of the outer AF. These defects result in a repair process, where granulation tissue is formed along with neovascularisation and concomitant ingrowth of nerve fibres (Melrose *et al*., 2002; Freemont *et al*., 1997; Aoki *et al*., 2006), and degenerated NP and AF cells produce neurotrophins that promote neurovascular growth (Purmessur *et al*., 2008). Although the AF has not fully lost its main function to withstand the hydrostatic pressure from the NP and to stabilise the segment, discogenic low back pain has a high likelihood to develop chronicity and often needs medical treatment. These discs are ideal candidates for cell-based therapies, as no relevant structural changes have occurred yet and restoration of disc height, intradiscal pressure and mechanical function is possible through regeneration of disc matrix. In addition, discogenic pain has an inflammatory component where introduction of metabolically active cells may have a beneficial, regulatory effect. On the other hand, these patients are presently not seen by spine surgeons but are treated by non-interventional specialists such as rheumatologists and general practitioners.

Prevention of adjacent segment disease (ASD)

ASD may be prevented by prophylactic treatment in the course of a surgical intervention for a symptomatic level. Frequently, discs adjacent to a segment that is fused for various indications show already signs of degeneration and are likely to also become symptomatic over time. Several attempts to reduce the incidence of ASD, such as total disc replacement, interspineous spacers or dynamic implants, are in clinical use. As these patients are already under treatment by spine surgeons and have a high risk for progression of the disease on adjacent levels, they represent ideal candidates for an attempt to restore the biological and biomechanical function of the adjacent disc. A phase I safety trial is on-going by a Japanese research group at Tokai University regarding cell therapy to prevent IVD degeneration in the adjacent disc. They target IVDs in patients in their 20s undergoing fusion surgery demonstrating mild to moderate degeneration in the IVD adjacent to the fusion level. They culture NP cells obtained from the IVD undergoing fusion surgery, and subsequently co-culture them with autologous MSCs isolated from the bone marrow to re-vitalise the NP cells. After re-vitalising, NP cells are injected under fluoroscopic guidance to the IVD adjacent to the fusion level. Preliminary results in 10 patients show that this technique seems to have no major issues regarding patient safety.

Prevention of post-discectomy syndrome

Partial discectomy for herniation is a common procedure; as disc herniation has a lifetime prevalence of 1-3 % and often affects active, working persons of 30-50 years of age, the socioeconomic impact due to medical treatment and long-term absence from work is enormous (Weinstein *et al*., 2006; Weber, 1994). Discectomy has been shown to be an effective treatment for acute disc herniation with regard to neurological symptoms, but fails to address the altered biomechanical properties of the segment and the resulting annular defect. In this situation, the surgeon faces the dilemma of how extensive a discectomy should be performed: if only the extruding material of the NP is resected, a relevant risk of recurrent disc herniation is well documented; however, if all or most of the NP is resected, there is also a significant chance that lost biomechanical function leads to instability or collapse of the segment (Moore *et al*., 1994; Kambin *et al*., 1995; Yorimitsu *et al*., 2001; Suk *et al*., 2001; Vucetic *et al*., 1997), increased disc degeneration and chronic low back pain (Barth *et al*., 2008). NP replacement or regeneration to restore the biomechanical function of the disc will only be successful in the presence of a functional AF that is able to restore function and withstand the physiological loading conditions (Veres *et al*., 2008; Thompson *et al*., 2000; Fazzalari *et al*., 2001). Synthetic and natural biomaterials capable of restoring functional biomechanics of the IVD are under development and offer promise for NP replacement, AF repair, and as an AF sealant (Iatridis *et al*., 2013). A successful regeneration of both the annulus and the nucleus must meet mechanical and biological compatibility requirements and is also strongly dependent on a sufficient anchorage of the regenerated tissue to the surrounding tissue. Cell-based therapies for annular repair may be helpful to overcome these barriers.

Total (biological) disc replacement for advanced disc degeneration

Total disc replacement by tissue engineered whole organs is of course an ambitious goal. Nevertheless, impressive advances have been made in whole disc replacement in rodents (Bowles *et al*., 2011). The patient population is large and heterogeneous, and a tissue engineered disc might be considered for specific indications in the future. So far unresolved barriers include the concomitant degeneration of the facet joints, ligaments and muscles.

Cell Therapy for Disc Repair: What are the Benefits?

Although the fate of the cells within the harsh environment of the disc with limited nutrient supply and transport of metabolites is unknown, there are several arguments for using cell-based therapies in attempts to regenerate the disc:

a) In degenerated discs, a reduced number of cells have been found in affected specimens that cannot be explained by limited nutrition and ageing alone.

b) Similarly, a loss of cells and matrix has been observed in disc herniations, further limiting the healing potential of the ruptured AF.

c) The effect of application of growth factors or other pharmaceuticals to regenerate the disc is temporally limited and may require several repetitive interventions or sustained release formulations, whereas the duration

of cell-based therapies depends on the survival and activity of the cells. Hence, a single stage application can have a long-lasting effect.

d) Cells can fulfil several functions, including matrix production, prevention of AF deformation, inflammation control, production of growth factors and prevention of angio- and neuro-genesis. Eventually, cells have the potential to interact with the resident cell population, regulate local homeostasis and attract additional cells (Illien-Junger *et al*., 2012).

The Need to Close the Gap towards Clinical Application

Selection of patients

Identification of suited patient populations is fundamental (see above). MRI is an ideal non-invasive screening method, and various protocols are available to quantitatively assess disc degeneration at early stages (Watanabe *et al*., 2007; Hoppe *et al*., 2012; Borthakur *et al*., 2011). Even though information on the nutritional status of the disc can be obtained (Rajasekaran *et al*., 2004; Benneker *et al*., 2005), to date these techniques are not yet applied in daily clinical use, mostly because of the lack of clinical consequences. Since the additional costs and effort to implement these modern sequences is moderate, they will most probably be implemented in daily routine once a regenerative treatment is available.

Standardisation of diagnostic parameters, outcome measurements and models

Until now standardised diagnostic parameters and outcome measurements for regeneration and repair are lacking. To monitor and compare effectiveness of regeneration strategies, such outcome measures need to be established on a clinical and investigational level. Efforts should be made to identify the best suited diagnostic method to quantify degenerative changes; ideally, non-invasive imageing methods such as quantitative MRI should be preferred that are widely available and can be used for both experimental and clinical purposes. Biochemically, assessment should focus on parameters that either can be reproduced on patients or are validated against the imageing techniques to be established. Similar to the lack of defined outcome parameters, *in viv*o and *in vitro* degeneration models need to be standardised to compare results of different treatments.

Cell type

Several sources have been investigated in cell therapy research. Optimal cell sources for clinical application are yet to be defined. While stem cells from bone marrow or adipose tissue show encourageing results in animal models, their senescence in aged adults is well documented. Embryonic allograft cells are appealing for their regenerative capacity, but their safe use is of concern. Autologous or allogeneic chondrocytes appear safe and effective in initial clinical trials (see above). Standardisation of procedures and longer term studies are needed to obtain reliable evidence about effectiveness of the treatment.

Delivery methods

Provocative discography has been shown to have a negative effect on discs, accelerating degeneration (Carragee *et al*., 2009), and this may arise due to altered mechanics around the AF defect or due to cytotoxicity of the delivery agent (Kang, 2010; Iatridis and Hecht, 2012). Alternative routes to deliver cells and other agents into the disc, keeping the AF intact, need to be developed (Vadala *et al*., 2013; Illien-Junger *et al*., 2012). Alternatively, proper repair of the AF may be an option (Guterl *et al*., 2013).

Large animal models and clinical studies

Many cell-based therapies have already shown promise based on *in vitro* or small animal models, and relevant pre-clinical screening in large animal models is required to accelerate translation. In animal models, degeneration is usually induced mechanically (stab injury, excessive torsion or compression, or removal of NP material), which is poorly comparable to the slow degenerative process in human discs. There is a need for establishment of large animal models that better mimic the disease process in humans.

Ultimately, in the light of approval from regulatory institutions (FDA), clinical studies for regenerative strategies need to be well designed with solid methodology. In particular, studies need to address the safety aspect of these treatments.

Conclusion

Due to the slow progressive nature of disc degeneration that involves cell senescence, increased catabolic activity and decreased matrix synthesis, cell therapy is an appealing approach to regenerate the intervertebral disc. This potential has already been demonstrated in pre-clinical studies, but there are also equally convincing amounts of scientific evidence demonstrating that further knowledge is required to overcome the hurdles. The most significant of these hurdles were identified in the workshop, hopefully providing some guidance for future research, in order to successfully translate this approach to the clinic for the benefit of patients suffering from this all too common and morbid condition.

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References

Acosta FL Jr, Metz L, Adkisson HD, Liu J, Carruthers-Liebenberg E, Milliman C, Maloney M, Lotz JC (2011) Porcine intervertebral disc repair using allogeneic juvenile articular chondrocytes or mesenchymal stem cells. Tissue Eng Part A **17**: 3045-3055.

Aoki Y, Akeda K, An H, Muehleman C, Takahashi K, Moriya H, Masuda K (2006) Nerve fiber ingrowth into scar tissue formed following nucleus pulposus extrusion in the rabbit anular-puncture disc degeneration model: effects of depth of puncture. Spine **31**: E774-E780.

Barth M, Weiss C, Thome C (2008) Two-year outcome after lumbar microdiscectomy *versus* microscopic sequestrectomy: part 1: evaluation of clinical outcome. Spine **33**: 265-272.

Benneker LM, Heini PF, Anderson SE, Alini M, Ito K (2005) Correlation of radiographic and MRI parameters to morphological and biochemical assessment of intervertebral disc degeneration. Eur Spine J **14**: 27-35.

Borthakur A, Maurer PM, Fenty M, Wang C, Berger R, Yoder J, Balderston RA, Elliott DM (2011) T1rho magnetic resonance imageing and discography pressure as novel biomarkers for disc degeneration and low back pain. Spine **36**: 2190-2196.

Bowles RD, Gebhard HH, Hartl R, Bonassar LJ (2011) Tissue-engineered intervertebral discs produce new matrix, maintain disc height, and restore biomechanical function to the rodent spine. Proc Natl Acad Sci USA **108**: 13106-13111.

Carragee EJ, Don AS, Hurwitz EL, Cuellar JM, Carrino JA, Herzog R (2009) Does discography cause accelerated progression of degeneration changes in the lumbar disc: a ten-year matched cohort study. Spine **34**: 2338-2345.

Coric D, Pettine K, Sumich A, Boltes MO (2013) Prospective study of disc repair with allogeneic chondrocytes presented at the 2012 Joint Spine Section Meeting. J Neurosurg Spine **18**: 85-95.

Crevensten G, Walsh AJ, Ananthakrishnan D, Page P, Wahba GM, Lotz JC, Berven S (2004) Intervertebral disc cell therapy for regeneration: mesenchymal stem cell implantation in rat intervertebral discs. Ann Biomed Eng **32**: 430-434.

Fazzalari NL, Costi JJ, Hearn TC, Fraser RD, Vernon-Roberts B, Hutchinson J, Manthey BA, Parkinson IH, Sinclair C (2001) Mechanical and pathologic consequences of induced concentric anular tears in an ovine model. Spine **26**: 2575-2581.

Freemont AJ, Peacock TE, Goupille P, Hoyland JA, O'Brien J, Jayson MI (1997) Nerve ingrowth into diseased intervertebral disc in chronic back pain. Lancet **350**: 178-181.

Ganey T, Hutton WC, Moseley T, Hedrick M, Meisel HJ (2009) Intervertebral disc repair using adipose tissue-derived stem and regenerative cells: experiments in a canine model. Spine **34**: 2297-2304.

Grad S, Alini M, Eglin D, Sakai D, Mochida J, Mahor S, Collin E, Dash B, Pandit A (2010) Cells and Biomaterials for Intervertebral Disc Regeneration, Morgan & Claypool, San Rafael, CA.

Guterl CC, See EY, Blanquer SB, Pandit A, Ferguson SJ, Benneker LM, Grijpma DW, Sakai D, Eglin D, Alini M, Iatridis JC, Grad S (2013) Challenges and strategies in the repair of ruptured annulus fibrosus. Eur Cell Mater **25**: 1-21.

Henriksson HB, Svanvik T, Jonsson M, Hagman M, Horn M, Lindahl A, Brisby H (2009) Transplantation of human mesenchymal stems cells into intervertebral discs in a xenogeneic porcine model. Spine **34**: 141-148.

Hiyama A, Mochida J, Iwashina T, Omi H, Watanabe T, Serigano K, Tamura F, Sakai D (2008) Transplantation of mesenchymal stem cells in a canine disc degeneration model. J Orthop Res **26**: 589-600.

Hohaus C, Ganey TM, Minkus Y, Meisel HJ (2008) Cell transplantation in lumbar spine disc degeneration disease. Eur Spine J **17 Suppl 4**: 492-503.

Hoppe S, Quirbach S, Mamisch TC, Krause FG, Werlen S, Benneker LM (2012) Axial T2* mapping in intervertebral discs: a new technique for assessment of intervertebral disc degeneration. Eur Radiol **22**: 2013-2019.

Iatridis JC, Hecht AC (2012) Does needle injection cause disc degeneration? News in the continuing debate regarding pathophysiology associated with intradiscal injections. Spine J **12**: 336-338.

Iatridis JC, Nicoll SB, Michalek AJ, Walter BA, Gupta MS (2013) Role of biomechanics in intervertebral disc degeneration and regenerative therapies: what needs repairing in the disc and what are promising biomaterials for its repair? Spine J **13**: 243-262.

Illien-Junger S, Pattappa G, Peroglio M, Benneker LM, Stoddart MJ, Sakai D, Mochida J, Grad S, Alini M (2012) Homing of mesenchymal stem cells in induced degenerative intervertebral discs in a whole organ culture system. Spine **37**: 1865-1873.

Kambin P, Cohen LF, Brooks M, Schaffer JL (1995) Development of degenerative spondylosis of the lumbar spine after partial discectomy. Comparison of laminotomy, discectomy, and posterolateral discectomy. Spine **20**: 599-607.

Kang JD (2010) Does a needle puncture into the annulus fibrosus cause disc degeneration? Spine J **10**: 1106-1107.

Meisel HJ, Siodla V, Ganey T, Minkus Y, Hutton WC, Alasevic OJ (2007) Clinical experience in cell-based therapeutics: disc chondrocyte transplantation. A treatment for degenerated or damaged intervertebral disc. Biomol Eng **24**: 5-21.

Melrose J, Roberts S, Smith S, Menage J, Ghosh P (2002) Increased nerve and blood vessel ingrowth associated with proteoglycan depletion in an ovine annular lesion model of experimental disc degeneration. Spine **27**: 1278-1285.

Moore AJ, Chilton JD, Uttley D (1994) Long-term results of microlumbar discectomy. Br J Neurosurg **8**: 319-326.

Orozco L, Soler R, Morera C, Alberca M, Sanchez A, Garcia-Sancho J (2011) Intervertebral disc repair by autologous mesenchymal bone marrow cells: a pilot study. Transplantation **92**: 822-828.

Purmessur D, Freemont AJ, Hoyland JA (2008) Expression and regulation of neurotrophins in the nondegenerate and degenerate human intervertebral disc. Arthritis Res Ther **10**: R99.

Rajasekaran S, Babu JN, Arun R, Armstrong BR, Shetty AP, Murugan S (2004) A study of diffusion in human lumbar discs: a serial magnetic resonance imageing study documenting the influence of the endplate on diffusion in normal and degenerate discs. Spine **29**: 2654-2667.

Sakai D, Mochida J, Yamamoto Y, Nomura T, Okuma M, Nishimura K, Nakai T, Ando K, Hotta T (2003) Transplantation of mesenchymal stem cells embedded in atelocollagen gel to the intervertebral disc: a potential therapeutic model for disc degeneration. Biomaterials **24**: 3531-3541.

Sakai D, Mochida J, Iwashina T, Watanabe T, Nakai T, Ando K, Hotta T (2005) Differentiation of mesenchymal stem cells transplanted to a rabbit degenerative disc model: potential and limitations for stem cell therapy in disc regeneration. Spine **30**: 2379-2387.

Sakai D, Mochida J, Iwashina T, Hiyama A, Omi H, Imai M, Nakai T, Ando K, Hotta T (2006) Regenerative effects of transplanting mesenchymal stem cells embedded in atelocollagen to the degenerated intervertebral disc. Biomaterials **27**: 335-345.

Sakai D, Nakamura Y, Nakai T, Mishima T, Kato S, Grad S, Alini M, Risbud MV, Chan D, Cheah KS, Yamamura K, Masuda K, Okano H, Ando K, Mochida J (2012) Exhaustion of nucleus pulposus progenitor cells with ageing and degeneration of the intervertebral disc. Nat Commun **3**: 1264.

Suk KS, Lee HM, Moon SH, Kim NH (2001) Recurrent lumbar disc herniation: results of operative management. Spine **26**: 672-676.

Thompson RE, Pearcy MJ, Downing KJ, Manthey BA, Parkinson IH, Fazzalari NL (2000) Disc lesions and the mechanics of the intervertebral joint complex. Spine **25**: 3026-3035.

Vadala G, Sowa G, Hubert M, Gilbertson LG, Denaro V, Kang JD (2012) Mesenchymal stem cells injection in degenerated intervertebral disc: cell leakage may induce osteophyte formation. J Tissue Eng Regen Med **6**: 348-355.

Vadala G, Russo F, Pattappa G, Schiuma D, Peroglio M, Benneker LM, Grad S, Alini M, Denaro V (2013) The transpedicular approach as an alternative route for intervertebral disc regeneration. Spine **38**: E319-E324.

Veres SP, Robertson PA, Broom ND (2008) Microstructure and mechanical disruption of the lumbar disc annulus: part II: how the annulus fails under hydrostatic pressure. Spine **33**: 2711-2720.

Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY, Ali MK, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Bahalim AN, Barker-Collo S, Barrero LH, Bartels DH, Basanez MG, Baxter A, Bell ML, Benjamin EJ, Bennett D, Bernabe E, Bhalla K, Bhandari B, Bikbov B, Bin AA, Birbeck G, Black JA, Blencowe H, Blore JD, Blyth F, Bolliger I, Bonaventure A, Boufous S, Bourne R, Boussinesq M, Braithwaite T, Brayne C, Bridgett L, Brooker S, Brooks P, Brugha TS, Bryan-Hancock C, Bucello C, Buchbinder R, Buckle G, Budke CM, Burch M, Burney P, Burstein R, Calabria B, Campbell B, Canter CE, Carabin H, Carapetis J, Carmona L, Cella C, Charlson F, Chen H, Cheng AT, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahiya M, Dahodwala N, Damsere-Derry J, Danaei G, Davis A, De LD, Degenhardt L, Dellavalle R, Delossantos A, Denenberg J, Derrett S, Des Jarlais DC, Dharmaratne SD, Dherani M, Diaz-Torne C, Dolk H, Dorsey ER, Driscoll T, Duber H, Ebel B, Edmond K, Elbaz A, Ali SE, Erskine H, Erwin PJ, Espindola P, Ewoigbokhan SE, Farzadfar F, Feigin V, Felson DT, Ferrari A, Ferri CP, Fevre EM, Finucane MM, Flaxman S, Flood L, Foreman K, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabbe BJ, Gabriel SE, Gakidou E, Ganatra HA, Garcia B, Gaspari F, Gillum RF, Gmel G, Gosselin R, Grainger R, Groeger J, Guillemin F, Gunnell D, Gupta R, Haagsma J, Hagan H, Halasa YA, Hall W, Haring D, Haro JM, Harrison JE, Havmoeller R, Hay RJ, Higashi H, Hill C, Hoen B, Hoffman H, Hotez PJ, Hoy D, Huang JJ, Ibeanusi SE, Jacobsen KH, James SL, Jarvis D, Jasrasaria R, Jayaraman S, Johns N, Jonas JB, Karthikeyan G, Kassebaum N, Kawakami N, Keren A, Khoo JP, King CH, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lalloo R, Laslett LL, Lathlean T, Leasher JL, Lee YY, Leigh J, Lim SS, Limb E, Lin JK, Lipnick M, Lipshultz SE, Liu W, Loane M, Ohno SL, Lyons R, Ma J, Mabweijano J, MacIntyre MF, Malekzadeh R, Mallinger L, Manivannan S, Marcenes W, March L, Margolis DJ, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGill N, McGrath J, Medina-Mora ME, Meltzer M, Mensah GA, Merriman TR, Meyer AC, Miglioli V, Miller M, Miller TR, Mitchell PB, Mocumbi AO, Moffitt TE, Mokdad AA, Monasta L, Montico M, Moradi-Lakeh M, Moran A, Morawska L, Mori R, Murdoch ME, Mwaniki MK, Naidoo K, Nair MN, Naldi L, Narayan KM, Nelson PK, Nelson RG, Nevitt MC, Newton CR, Nolte S, Norman P, Norman R, O'Donnell M, O'Hanlon S, Olives C, Omer SB, Ortblad K, Osborne R, Ozgediz D, Page A, Pahari B, Pandian JD, Rivero AP, Patten SB, Pearce N, Padilla RP, Perez-Ruiz F (2012) Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet **380**: 2163-2196.

Vucetic N, de BE, Svensson O (1997) Clinical history in lumbar disc herniation. A prospective study in 160 patients. Acta Orthop Scand **68**: 116-120.

Watanabe A, Benneker LM, Boesch C, Watanabe T, Obata T, Anderson SE (2007) Classification of intervertebral disk degeneration with axial T2 mapping. Am J Roentgenol **189**: 936-942.

Weber H (1994) The natural history of disc herniation and the influence of intervention. Spine **19**: 2234-2238.

Weinstein JN, Lurie JD, Olson PR, Bronner KK, Fisher ES (2006) United States' trends and regional variations in lumbar spine surgery: 1992-2003. Spine **31**: 2707-2714.

Yorimitsu E, Chiba K, Toyama Y, Hirabayashi K (2001) Long-term outcomes of standard discectomy for lumbar disc herniation: a follow-up study of more than 10 years. Spine **26**: 652-657.

Yoshikawa T, Ueda Y, Miyazaki K, Koizumi M, Takakura Y (2010) Disc regeneration therapy using marrow mesenchymal cell transplantation: a report of two case studies. Spine **35**: E475-E480.

Zhang Y, Drapeau S, Howard SA, Thonar EJ, Anderson DG (2011) Transplantation of goat bone marrow stromal cells to the degenerating intervertebral disc in a goat disc injury model. Spine **36**: 372-377.

Editor's Note: All comments/questions by the reviewers were answered by making changes in the text. There is hence no Discussion with Reviewers section.